Research Paper | Education | E-ISSN No : 2454-9916 | Volume : 10 | Issue : 5 | May 2024

EVALUATION OF PHYTOCHEMICALS, MOSQUITO LARVICIDAL AND ALLELOPATHIC POTENTIAL OF AGERATUM CONYZOIDES L.

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ABSTRACT

Ageratum conyzoides, commonly known as "Billygoat Weed" or "Whiteweed," is a medicinal plant with widespread distribution and recognized for its traditional uses. This study aims to investigate the allelopathic potential of *A. conyzoides*, assess its mosquito larvicidal activity, and conduct a preliminary phytochemical screening to identify bioactive compounds. The allelopathic impact will be evaluated through germination assays and seedling growth inhibition experiments, shedding light on the potential of *A. conyzoides* in natural weed control strategies. Simultaneously, the mosquito larvicidal activity will be examined by evaluating the efficacy of *A. conyzoides* extracts against mosquito larvae, contributing to the exploration of environmentally friendly mosquito control approaches. Preliminary phytochemical screening will involve the identification of primary and secondary metabolites present in *A. conyzoides*, providing a foundation for further isolation and characterization of potential pharmacologically active compounds. The comprehensive findings from this study will contribute to a better understanding of the multifaceted ecological roles of *A. conyzoides* and its potential applications in weed management and vector control.

KEYWORDS: *Ageratum conyzoides* L., Phytochemicals, Allelopathic Potential, Larvicidal Activity

INTRODUCTION

Ageratum conyzoides L. is an annual herbaceous plant with worldwide distribution, particularly in the subtropical and tropical regions, which belongs to the family Asteraceae. *Ageratum conyzoides*, commonly known as Billygoat weed, is a tropical plant that has garnered scientific interest due to its diverse phytochemical constituents and associated biological activities. Native to Central America, this invasive species has spread globally, often considered a weed in agricultural and natural ecosystems. Research into *Ageratum conyzoides* has revealed a rich array of secondary metabolites with promising applications.

Studies have identified significant mosquito larvicidal properties of *Ageratum conyzoides* (Rajkumar and Jebanesan, 2005), highlighting its potential as a natural alternative in vector control strategies. Additionally, the plant exhibits allelopathic effects, influencing the growth and development of surrounding flora through the release of bioactive compounds (Singh et al., 2003). These attributes underscore the ecological impact and utility of *Ageratum conyzoides* in sustainable agricultural practices and pest management.

Recent evaluations have focused on isolating and characterizing these phytochemicals to better understand their mechanisms and optimize their use in various biocontrol applications (Kong et al., 2019). This multifaceted potential positions *Ageratum conyzoides* as a valuable subject for ongoing phytochemical, ecological, and entomological research.

However, *Ageratum conyzoides* L. has beneficial uses in traditional medicine, its invasive tendencies in certain ecosystems raise concerns about its impact on native flora. Researchers continue to study its ecological interactions, chemical composition, and potential medicinal applications, balancing the plant's cultural significance with the need for sustainable management practices in areas where it is considered invasive.

MATERIALS AND METHODS

Collection and Drying of *Ageratum conyzoides* Entire Plant:
Collect *Ageratum conyzoides* plants, dry, and preserve them as herbarium. Select fresh and healthy leaves, wash thoroughly, and dry in shade for 3-4 weeks. Powder the dried plant materials using an electric grinder and store separately.

Preparation of Aqueous Extract of *Ageratum conyzoides*:
Mix 25g of sample powder with 250ml of distilled water in a beaker and water bath. Filter the crude extract using Whatman No: 1 filter paper. Prepare different concentrations (100%, 75%, 50%, 25%) of the extract using distilled water.

Germination Study:
Transfer selected *Abelmoschus esculentus* seeds into petri dishes with moistened filter paper. Add different extract concentrations to each dish. Maintain petri dishes on germination tables at room temperature (25-30ºC). Record daily germination by visual counting for eight days.

Control of the Experiment:
Maintain a control group with seeds moistened with distilled water.

Germination Percentage (G.P):
Compute G.P using the formula: G.P = (No. of seeds germinated / Total no. of seeds) × 100.
Testing Larvicidal Activity

Materials Required:
Test organism: Approximately 45 mosquito larvae of 3rd or 4th instars. Glass wares and other requirements: Test tubes, test tube stand, beakers, glass rods, filter paper, mortar and pestle, weighing machine, electric grinder, cello tape, labels, etc.

Method:
Collection and Identification of Mosquito Larvae. Collect larvae from stagnant water and identify them. Use 45 larvae of 3rd or 4th instars for the bioassay.

Preparation of Plant Extract:
Powder shade-dried leaves of *Ageratum conyzoides* using an electric grinder. Weigh different amounts of powder and add distilled water in test tubes. Add larvae to each tube and record mortality after 24 hours.

Larval Bioassay:
Follow WHO guidelines for larval bioassays. Prepare stock solutions of solvent extracts and test their larvicidal activities.

Preliminary Phytochemical Screening
Preparation of Plant Extract:
Shade dry and crush fresh whole plant of *Ageratum conyzoides*. Dissolve leaf powder in distilled water and methanol for solvent preparation.

Detection of Primary Metabolites:
Proteins: Perform Xanthoproteic and Biuret tests.
Carbohydrates: Perform Molisch's and Benedict's tests.

Detection of Secondary Metabolites:
Alkaloids: Perform Mayer's and Wagner's tests.
Flavonoids: Perform NaOH and H2SO4 tests.
Phenols: Perform $FeCl_3$ and lead acetate tests.
Terpenoids: Perform Liebermann–Burchard and Salkowski tests.
Steroids: Perform Salkowski and Liebermann–Burchard tests.
Tannins: Perform $FeCl_3$ and lead acetate tests.
Saponins: Perform Foam and Froth tests.
Glycosides: Perform Conc. H2SO4 and Salkowski tests.
Quinones: Perform HCl and H2SO4 tests.
Coumarins: Perform FeCl3 and fluorescence tests.
Phlobetannin: Perform HCl test.
Anthocyanin: Perform HCl test.

RESULT AND DISCUSSION

Allelopathic Effect
The allelopathic effect of dried leaf extracts of *Ageratum conyzoides* on seed germination of *Abelmoschus esculentus* (L.) Moench was investigated. Germination was inhibited with increasing concentration of the extract. The number of sprouted seeds after breaking the dormancy was higher in the control and 25% extract concentration. None were germinated in the 50%, 75% and 100% extract concentration. Observations were recorded for eight days. The germination was observed on the Day 3. In the Control sample, all the seeds were germinated on the third day. Five 25% extract treated seeds were also germinated and no seeds were germinated in the 50% concentration.

Table 1:
Effect of dried plant extracts of *Ageratum conyzoides* on seed Germination.

Germination percentage = No. of seeds germinated / Total No. of seeds x 100

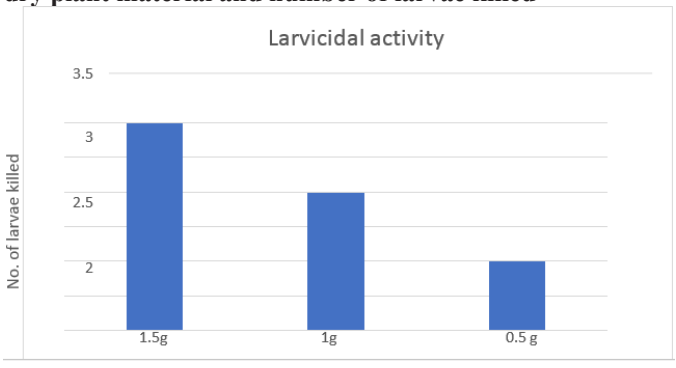
Sl No.	Extract concentration	No. of seeds germinated	Germination percentage
1	Control	10	100%
2	25%	5	50%
3	50%	0	0%
4	75%	0	0%
5	100%	0	0%

Larvicidal Activity
The dry plant extract of *Ageratum conyzoides* screened against *Aedes aegypti* for their larvicidal efficacy. In this present study, the results indicate that mortality values significantly increased depending on the concentration of plant extract. The mortality rate of *Aedes aegypti* is summarized in the table.

Table 2: The mortality rate of *Aedes aegypti* larvae by various weights of plant extracts.

Material	No. of larvae killed by the extracts at various various weights (in g) [after 6 hours]		
	0.5g	1g	1.5g
Dry leaf extract	1	2	3

GRAPH 1: showing the relationship between weight of the dry plant material and number of larvae killed



Preliminary Phytochemical Screening
Preliminary phytochemical screening of *Ageratum conyzoides* leaves showed the presence of both primary and secondary metabolites. Compounds like carbohydrates, proteins, Alkaloids, Flavonoids, Phenols, Terpenoids, Steroids, Tannins, Saponins, Glycosides, Quinones, and Coumarins were present in the aqueous extract. The methanolic extract showed the

presence of compounds like carbohydrates, proteins, Alkaloids, Flavonoids, Phenols, Terpenoids, Steroids, Tannins, Glycosides, Quinones, and Coumarins.

Table 3: Phytochemical screening of primary metabolites in *Ageratum conyzoides* plant extract.
+ indicates presence, - indicates absence

Sl.No.	Primary metabolite	Name of test	Water extract	Methanol extract
1	Carbohydrates	Molisch's test	+	+
		Benedict's test	+	+
2	Proteins	Xanthopro-teic test	+	+
		Biuret test	+	+

Table 4: Phytochemical screening of secondary metabolites in *Ageratum conyzoides* plant extract.

Sl.No.	Secondary metabolite	Name of test	Water	Methanol
1	Alkaloids	Mayer's test	+	+
		Wagner's test	+	+
2	Flavonoids	NaOH test	+	+
		H_2SO4 test	+	+
3	Phenols	$FeCl_3$ test	+	+
		Lead acetate test	+	+
4	Terpenoids	Liebermann-Burchard test	+	+
		Salkowski test	+	+
5	Steroids	Salkowski test	+	+
		Liebermann-Burchard test	+	+
6	Tannins	$FeCl_3$ test	+	+
		Lead acetate test	+	+
7	Saponins	Foam test	+	-
		Froth test	+	-
8	Glycosides	Conc. H_2SO4 test	+	+
		Salkowski test	+	+
9	Quinones	HCl test	+	+
		H_2SO4test	+	+
10	Coumarins	$FeCl_3$test	+	+
		Fluorescence test	+	+
11	Phlobetannin	HCl test	-	-
12	Anthocyanin	HCl test	-	-

CONCLUSION

The study on allelopathy, phytochemistry, and larvicidal activity of *Ageratum conyzoides* aimed to investigate the allelopathic potential of *Ageratum conyzoides* extracts on seed germination of certain plant species, analyze its phytochemical constituents, and evaluate its larvicidal activity against mosquito larvae. The allelopathic effects were assessed through various bioassays. And the study showed that, after 3 days the seeds in control started to germinate and at day 8 all the seeds in control was germinated and 5 seeds in 25% plant extract was germinated and in 50%,75% and 100% of plant extract no seeds were germinated. It shows that as the concentration of plants extract increases, the percentage of seed germination decreases.

The phytochemical analysis involved the identification of primary and secondary metabolites present in the plant extracts. Primary metabolites, carbohydrates and proteins are present in Ageratum conyzoides plant extract. Secondary metabolites, Alkaloids, Flavonoids, Phenols, Terpenoids, Steroids, Tannins, Glycosides, Quinones, and Coumarins are present in both of plant extract in water and methanol. Saponins are present in water extract but absent in methanol extract. Phlobetannin and Anthocyanin are both absent in water and methanol plant extract.

The larvicidal activity was determined by subjecting mosquito larvae to different concentrations of Ageratum conyzoides extracts. The findings revealed that as the concentration of plant extract increases, the number of larvae died also increases.

The significant allelopathic potential, presence of various phytochemicals, and notable larvicidal activity against mosquito larvae, highlighting the plant's potential as a natural source for bioactive compounds with agricultural and public health implications.

In conclusion, *Ageratum conyzoides* exhibits allelopathic effects on seed germination and seedling growth of certain plant species, indicating its potential for weed management in agricultural ecosystems. Phytochemical analysis revealed the presence of diverse secondary metabolites, which could contribute to its allelopathic and larvicidal activities. Furthermore, the significant larvicidal activity against mosquito larvae suggests its potential application in mosquito control programs for vector-borne disease prevention. Overall, these findings underscore the importance of further exploration of *Ageratum conyzoides* as a source of bioactive compounds for agricultural and public health purposes.

ACKNOWLEDGEMENT

We express our heartfelt gratitude to the Principal and Management of Kottakkal Farook Arts & Science College for their unwavering support and the excellent facilities provided, which were instrumental in the successful completion of our work.

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